SEN IT'S APPLICATION IN CARBON SEQUESTRATION

RenuYadav, Darshika Nigam, Abhash, VicseVerma, Udita Tiwari*
Department of Biochemistry, School of life Sciences, Khandari Campus,
Dr. B. R. Ambedkar University, Agra.

Corresponding Author:
Dr. Udita Tiwari
Department of Biochemistry, School of life Sciences, Khandari Campus,
Dr. B. R. Ambedkar University, Agra.

Abstract: The world is now dealing with various environmental problems where nanobiotechnology is emerging as a new avenue for safer and cleaner environment. The studies on CO_2 sequestration are being pursued all across the world. Many research works are focused on the development of single enzyme nanoparticles of enzymes were targeted for environmental bioremediation. This work is used to improve the stability and the active life of biocatalyst by using the approach of stabilization and immobilization, either singly or in combination. There are researches on the development of synthesis of single enzyme nanoparticles of carbonic anhydrase (SEN-CA). Individual enzyme molecules are stabilized within a biopolymeric silica network of nanometer scale thickness. A key result is that stabilization of the enzyme activity was achieved with minimal substrate mass transfer limitation compared to enzyme entrapped in larger scale materials. The development of stabilized SEN as soluble individual SEN particles also provides the opportunity to further immobilized on to the different matrices. These SEN provides major advantages such as stabilization of the activity, reduced mass-transfer limitation relative to large-scale immobilizations, and process ability into additional forms including hierarchical architectures. There are reports on biomimetic approach for sequestration of CO_2into calcium carbonate using stabilized/immobilized CA as a non-site scrubber. Carbon sequestration by biological means will produce safe, environmental friendly products like calcium carbonate. It will be a permanent method for disposing CO_2which shall make our environment cleaner, greener and sustainable.

Key Words: environment, nanotechnology, immobilization, single enzyme nanoparticles, carbon dioxide

1. Introduction:

Carbon dioxide (CO_2) is one of the important pollutants responsible for the green house effect. Point sources of CO_2 emission is from industries such as fossil fuel fired power plants, steel production, natural gas purification and petrochemical sectors. There is an urgent need to explore ways to reduce the emissions of CO_2 to the atmosphere in order to mitigate the adverse climatic and environmental impacts. Several chemistry, chemical engineering and biological approaches are being used singly or in combination for CO_2 sequestration. In this connection, efforts are being made to mimic the reaction for fixation of anthropogenic CO_2 into calcium carbonate using carbonic anhydrase (CA, carbonate hydrolyase, EC 4.2.1.1, a zinc containing enzyme) as a biocatalyst.

Mineralization of CO_2 generally involves five steps.

$$CO_2\,(g) \longleftrightarrow CO_2\,(aq) \quad (1)$$

This reaction is rapid. The aqueous CO_2 may then react either with water or, at high pH with hydroxyl ions.

$$CO_2\,(aq) + H_2O \longleftrightarrow H_2CO_3 \quad (2)$$

$$H_2CO_3 \longleftrightarrow H^+ + HCO_3^- \quad (3)$$

$$HCO_3^- \longleftrightarrow CO_3^- + H^+ \quad (4)$$

$$Ca^{2+} + CO_3^- \longleftrightarrow CaCO_3 \quad (5)$$

Calcium carbonate can be precipitated from aqueous solution, given a suitable saturation of calcium and carbonate ions, and so the issue to be addressed is to produce carbonate ions rapidly from CO_2 and H_2O, a process which first requires the formation of bicarbonate ions. CA is being employed to accelerate the rate of hydration of CO_2 to form carbonate ions and proton which is further converted into calcium carbonate. This is safe, environmentally benign and permanent method for disposing CO_2 is termed as Biomimetic sequestration of carbon dioxide. The resulting biomimetic sequestration system would offer several advantages, including:

- No costly CO_2 concentration and transportation steps.

- A safe, stable, environmentally benign product.
- An environmentally friendly process, performed in aqueous solution at near ambient temperatures.
- A site-specific solution to CO_2 sequestration.

2. CA and Carbon Sequestration:

CA is ubiquitously found in prokaryotes as well as eukaryotes. It has the highest turnover number of all known enzymes ($1.4 \times 10^6 s^{-1}$ at 25 ^oC for human CA isozyme II),The enzyme plays a role in CO_2 fixation in photoautotrophic, chemoautotrophic and heterotrophic prokaryotes. In the presence of carbonic anhydrase enzyme, the mechanism of hydration of CO_2 changes and the rate-limiting step, which has the slowest reaction rate, is eliminated and therefore, the overall reaction rate is enhanced dramatically.

Bond et al. (2001) developed an integrated system in which they used the enzyme CA to accelerate the hydration of CO_2 for converting it into mineral carbonate. They also investigated the effect of other chemical species (NOx, SOx) on the bovine carbonic anhydrase activity. NOx and SOx is very important in the flue gases. According to their work, high concentration level of NOx (>0.05M) and SOx (>0.005M) inhibited the enzyme. In another study, they have also immobilized the CA on three different matrices: acrylamide, alginate, and chitosan-alginate. Among them, alginate and chitosan-alginate matrices shown to give better enzyme activity than acrylamide (Bond et al., 2001).

Mirjafari et al. (2007) investigated the application of the CA to enhance the hydration of CO_2 in the solution and its precipitation in the form of calcium carbonate. They reported that the rate of hydration of CO_2 increased in the presence of both the enzyme concentration and temperature. They also reported that increase in the temperature caused increase in calcium carbonate formation and also the enzyme activity was not influenced by the pH of the reaction mixture. Based on the experimental results, they determined the activation energy and catalytic rate constant of carbonic anhydrase for CO_2 substrate as 700.91 cal/mol and 0.65 s-1, respectively.

Liu et al. (2005) studied the precipitation of $CaCO_3$ from produced waters in the presence of the CA enzyme. They reported that precipitation of calcium carbonate occurred much more quickly in presence of bovine carbonic anhydrase. They also investigated the effect of temperature on precipitation time. They have shown that the increases in temperature accelerated the precipitation for both enzymatic and control.

Favre et al. (2009) reported the detailed study of formation of calcium carbonate by CA. They used bovine CA for their study. They reported that the initial precipitation rate of the solid depended on the capacity and strength of the initial buffer stock solution used and on the quantity of enzyme. An acceleration of the formation rate of $HCO3^-$ anions, either with the buffer or an increasing mass of enzyme, may decrease the pH at a rate so fast that the overall precipitation of $CaCO_3$(s) rate may itself decrease. They have also characterized the calcium carbonate precipitate by using SEM and XRD. They reported that at higher pH two phases of $CaCO_3$ i.e. calcite and vaterite was observed while at lower pH, the formation of calcite was favoured.

Bhattacharya et.al. (2004) developed novel spray reactors with immobilized CA for solubilization and concentration of carbon dioxide. They immobilized the CA in different porous matrices and applied water spray, instead of solution phase, to enhance the solubility of CO_2 so that an enhancement in the CO_2 capture was observed without any significant pressure drop or backpressure in the emission stream with facilitated mass transfer to aqueous phase.

3. Enzyme Immobilization Technology

It describes enzymes physically confined at or localized in a certain region of space with retention of catalytic activity and which can be used repeatedly and continuously. In general, immobilized biocatalysts are more stable and easier to handle compared with their free counterparts (Doaa et al., 2009; Tian et al., 2009).

3.1 Method of Catalyst Immobilization

Various methods have been developed for the immobilization of biocatalysts, which are being used extensively today. A wide range of support materials has also been employed for enzyme immobilization. The support type can be classified according to their chemical composition, such as organic or inorganic supports.

3.1.1 Adsorption:

Basically, the enzyme is attached to the support material by noncovalent linkages and does not require any preactivation step of the support. The interactions formed between the enzyme and the support material will be dependent on the existing surface chemistry of the support and on the type of amino acids exposed at the surface of the enzyme molecule (Panzavolta et al,. 2005) Enzyme immobilization by adsorption involves, normally, weak interactions between the support and the enzyme such as ionic or hydrophobic interactions, hydrogen bonding, and van der Waals forces.

3.1.2 Ionic Binding:

Immobilization via ionic binding is based, mainly, on ionic binding of enzyme molecules or active molecule to solid supports containing ionic charges. In this method, the amount of enzyme bound to the carrier and the activity after immobilization depends on the nature of the carrier.

3.1.3 Covalent Bonding:
The covalent bonding method is based on the binding of enzymes, or other active molecules, to a support or matrix by means of covalent bonds. The bond is normally formed between a functional group present on the support surface and amino acid residues on the surface of the enzyme (Quiocho et al., 1964).

3.1.4 Crosslinking:
The crosslinking method is based on the formation of covalent bonds between the enzyme or active molecules, by means of bi- or multifunctional reagents. The individual biocatalytic units (enzymes, organelles, whole cells) are joined to one another with the help of bi- or multifunctional reagents (e.g., glutaraldehyde, glyoxal, diisocyanates, hexamethylenediisocyanate, toluene diisocyanate, etc.). Enzyme crosslinking involves normally the amino groups of the lysine but, in occasional cases, the sulfhydryl groups of cysteine, phenolic OH groups of tyrosine, or the imidazol group of histidine can also be used for binding (Quiocho et al. 1964, Stclair et al. 1992).

3.1.5 Entrapment and Encapsulation:
The entrapment method for immobilization consists of the physical trapping of the active components into a film, gel, fiber, coating, or microencapsulation. This method can be achieved by mixing an enzyme or active molecule with a polymer and then crosslinking thepolymer to form a lattice structure that traps the enzyme (Zhao etal.,2003) .

3.2 Supports Used in the CA Immobilization:
There are number of materials designed for the immobilization of CA depending on its and matrices chemical and physical properties (Wang et al.2008 ; Wang et al. 2009 ; Chong et al. 2004;Gemeiner et al. 1992 ; Luckarift et al. 2004 ; Sun et al. 2006 ; Wang et al. 2006 ;Wang et al. 2009).

Prabhu et al.(2001) reported the partially purified bacterial CA isolated from three different microorganisms viz*P. fragi*, *M. lylae* and *Bacillus pumilus*. These microorganisms have been isolated from different regions, including soils around CaCO3 kilns and soil from around a thermal power industry, and have been used in conversion of CO2 to mineral carbonates for sequestration of carbondioxide. P. fragi shows a significantly high CO2 sequestration capacity of 27.33 mg of CaCO3/mg of CA in 15 min compared to *B. pumilus* and *M. lylae*.
A wide array of materials have been developed at NEERI such as chitosan based, bioceramic and mesoporous based materials for immobilization of CA. They have been tested for p-NPA assay and have also been further utilized for evaluating the CO2 sequestration capacity (Yadav et al. 2010; Prabhu et al . 2011;Wanjari et al . 2012; Wanjari et al . 2011 ; Yadav et al . 2012 ; Rayalu et al . 2013)

3.3Single Enzyme Nanoparticles of Carbonic Anhydrase:
Enzymes are natural biocatalysts of nanometer scale, which are being highly specific and effective under ambient conditions, show great promise in various industrial applications including in environmental monitoring, enzymatic carbon sequestration, laundry detergents, pharmaceuticals, biosensor, bioremediation, chemical conversions, biobleaching, biofuels cells, proteomics, bio-marker analysis and in virus detection etc. The major drawback in their use is their short half life period. Improvements in enzyme stability can enable further practical applications. Various methods have been reported to improve catalytic stability of enzymes such as immobilization, modification, genetic modification and medium engineering; this also includes the recent developments with cross-linked enzyme crystals (CLECs) and cross- linked enzyme aggregates (CLEAs) (Fagain et al., 2003; Shanbag et. al., 2016; Khameneha, 2017). In more detail, we will discuss the enzyme stabilization using nanoparticles, nanofibers, nanoparticles prepared via sol–gel encapsulation and a new enzyme composite of nanometer scale (SENs) was developed.
A growing interest has been shown in using nanoparticles as carriers for enzyme immobilization (Jia et al. 2003). The effective enzyme loading on nanoparticles could be achieved up to 10% wt due to a large surface area per unit mass of nanoparticles along with minimum diffusional limitation (Chen et al. 2001). Nanofibers provide a large surface area for the attachment or entrapment of enzymes and the enzyme reaction. In the case of porous nanofibers, they can still reduce the diffusional path of substrate from the reaction medium to the enzyme active sites due to the reduced dimension in size.
In the recent years, a new enzyme composite of nanometer scale that is “Single Enzyme Nanoparticles (SENs)” Kim et al. (2006) had been developed. In SENs, an enzyme molecule is modified by enclosing it in a cage formed with a porous organic / inorganic structure of less than a few nanometers thick. SEN of chymotrypsin and trypsin have been endorsed with great advantages such as increased stability and activity, high surface area, performance at high temperatures, stability to denaturation and aggregation (Yan et al., 2006), with minimal mass-transfer limitation on substrates and improved half life (Kim et al.2006; Kim et al. 2003).SENs can be further immobilized into mesoporous silica with a large surface area, providing a hierarchical approach for stable immobilized enzyme systems.

SadhanaRayalu group at NEERI had develop single enzyme nanoparticles (SENs) and multiple enzymes macro-particles (MEMs) from enzymes like tyrosinase and CA, which functions in enhanced humification and for increased CO_2 sequestration (Rayalu et al.,2013). Single enzymes are to be stabilized with chitosan, biopolymer and subsequently treated with silicates to form organic-inorganic hybrid matrix. This is in contract to the existing methods reported by Kim et. al. (2003) wherein several harsh steps are involved to develop a polymer around the enzyme. This approach of improving enzyme stability is to be tested for the application of enhanced humification and biomimetic CO2 sequestration using tyrosinase and carbonic anhydrase. A new and simple process has been developed for stabilization of enzymes with minimal limitation of mass transfer as compared to other entrapment techniques reported. Individual enzyme molecules are stabilized within a biopolymeric silica network of nanometer scale thickness called as "Single Enzyme Nanoparticles of Carbonic Anhydrase (SEN-CA)". The soluble form of SEN-CA facilitates its processing into other forms including immobilized SEN-CA etc. The synthesis protocol is versatile and can be employed for stabilizing other enzymes (Rayalu et al.,2013).

4. Conclusion:

To reduce the atmospheric CO2 level extensive efforts are being made worldwide by converting into begin product CaCO3 through biomimetic CO2 sequestration using immobilized and stabilized CA. This review paper reports unprecedented enhancement of biomimetic carbonation using SEN-CA of 3,555 mg of CaCO3/mg of CA coupled with improved storage stability up to 30 days for SEN-CA promises the possibility of a commercially feasible biomimetic process for carbonation reaction. These findings and hypothesis tailored together may lead to some revolutionary developments in carbon sequestration with emphasis on valorization.

Acknowledgement:

The author Dr. RenuYadav would take the opportunity to sincerely acknowledge Dr. SadhanaRayalu , deputy director and head of environmental material division and director, NEERI, for providing the research facilities and for constant support and encouragement.

References :

1. Bond, G.M., Stringer, J., Brandvold, D.K., Simsek, F.A., Medina, M., Egeland, G., 2001.Development of Integrated System for Biomimetic CO_2 Sequestration Using the Enzyme Carbonic Anhydrase**.** Energy & Fuels**,** 15:309-316.
2. Bond, G.M., Medina, M.G., Stringer, J., 2001. Eighteenth Annual International Pittsburgh Coal Conference Proceedings. Newcastle, NSW, Australia.
3. Mirjafari, P., Asghari, K., Mahinpey, N., 2007. Investigating the application of enzyme carbonic anhydrase for CO2 sequestration purposes. Industrial&Engineering Chemistry Research**,** 46: 921–926.
4. Liu, N., Bond, G.M., Abel, A., McPherson, B.J., Stringer, J., 2005. Biomimetic sequestration of CO2 in carbonate form: role of produced waters and other brines. Fuel Process Technology**,** 86: 1615–1625.
5. Favre, N., Christ, M.L., Pierre, C.P.,2009Biocatalytic capture of CO2 with carbonic anhydrase and its transformation to solid carbonate. Journal of MolecularCataylsis B: Enzymatic**,** 60: 163-170.
6. Bhattacharya, S., Nayak, A.,Schiavone, M., Bhattacharya, S.K. 2004.Solubilization and concentration of carbon dioxide: Novel sprays reactors with immobilized carbonic anhydrase. Biotechnology Bioengineering, 86: 37-46.
7. Doaa, A.R., Mahmoud,andWafaa, A. Helmy., 2009.Potential Application of Immobilization Technology in Enzyme and Biomass Production (Review Article). Journal of Applied Sciences Research, 5(12): 2466-2476.
8. Xie, T., Wang, A., Huang, L.,Haifeng Li, Zhenming Chen, Qiuyan Wang and Xiaopu Yin., 2009. Recent advance in the support and technology used in enzyme immobilization. African Journal of Biotechnology; 8 (19): 4724-4733.
9. Mansour, E.H. and Dawoud, F.M., 2003. Immobilization of invertase on celite and on polyacrylamide by an absorption procedure. Journal of the Science of Food and Agriculture; 83:446–450.
10. Panzavolta, F., Soro, S., D'Amato, R, Palocci, C., Cernia, E, Russ, M.V., 2005.Acetylenic polymers as new immobilization matrices for lipolytic enzymes. Journal of Molecular Catalysis B: Enzymatic; 32, 67–76.
11. Quiocho, F.A., Richards, F.M., 1964. Intermolecular cross linking of a protein in the crystalline state: carboxypeptidase-A. Proceedings of National Academy of. Science. USA; 52(3): 833-839.
12. Stclair, N.L., Navia, M.A., 1992. Cross-linked enzyme crystals as robust biocatalysts. Journal of the American Chemical Society, 114(18): 7314-7316.
13. Zhao, S. X., Bao, X.Y., Guo, W, and Lee, F.L., 2003.Immobilizing catalysts on porous materials. Materials Today, 9: 32-39.
14. Wang, A.M., Wang, H., Zhou, C., Du, Z.Q., Zhu, S.M., Shen SB., 2008. Ag-induced efficient immobilization of papain on silica spheres. Chinese Journal of Chemical Engineering,16 (4): 612-619.

15. Wang, A.M., Zhou, C., Liu, M.Q., Du,Z.Q, Zhu SM, Shen, S.B., Ouyang, P.K. 2009, Enhancement of microwave-assisted covalent immobilization of penicillinacylase using macromolecular crowding and glycine quenching. Journal of Bioscience and Bioengineering, 107: 219-224.
16. Chong, A.S.M., Zhao, X.S., 2004, Design of large-pore mesoporous materials for immobilization of penicillin G acylase biocatalyst. Catalysis Today, 93-95: 293-299.
17. Gemeiner, P., 1992, Materials for enzyme engineering. In enzyme engineering- immobilized Biosystems. England: Ellis Horwood Limited, pp. 13-119.
18. Luckarift, H.R., Spain, J.C., Naik, R.R., Stone, M.O., 2004, Enzyme immobilization in a biomimetic silica support. Nature Biotechnology; 22 (2): 211-213.
19. Sun, J.M., Zhang, H., Tian, R.J., Ma .D, Bao, X.H., Su, D.S., Zou, H.F., 2006, Ultrafast enzyme immobilization over large-pore nanoscalemesoporous silica particles. Chemical Communication, (12): 1322-1324.
20. Wang. P., 2006. Nanoscale biocatalyst systems, Current Opinion in Biotechnology, 17 (6): 574-579.
21. Wang, Z.G., Wan L.S., Liu, Z.M., Huang, X.J., Xu, Z.K., 2009. Enzyme immobilization on electrospun polymer nanofibers: An overview Journal of Molecular Catalysis B: Enzymatic, 56 (4): 189-195.
22. Prabhu, C., Wanjari, S., Puri, A., Bhattacharya, A., Pujari R, Yadav R, Das S, Labhsetwar N, Sharma A, Satyanarayanan T, Rayalu S., 2011,Region-Specific Bacterial Carbonic Anhydrase for Biomimetic Sequestration of Carbon Dioxide Energy Fuels 25:1327.
23. Yadav, R., Wanjari, S., Prabhu, C., Kumar, V., Labhsetwar, N., Satyanarayanan, T., Kotwal, S., Rayalu, S., 2010. Immobilized Carbonic Anhydrase for the Biomimetic Carbonation ReactionEnergy Fuels, 24:6198.
24. Prabhu, C., Valechha, A., Wanjari, S., Labhsetwar, N., Kotwal. S., Satyanarayanan, T., Rayalu, S., 2011, Carbon composite beads for immobilization of carbonic anhydrase Journal of Molecular Catalysis B: Enzymatic 71:71.
25. Wanjari, S., Prabhu, C., Satyanarayana, T., Vinu, A., Rayalu, S., 2012,Immobilization of carbonic anhydrase on mesoporousaluminosilicate for carbonation reaction,MicroporousMesoporous Mater 160:151.
26. Wanjari, S., Prabhu, C., Yadav. R., Satyanarayana, T., Labhsetwar, N., Rayalu, S., 2011Nanobiocatalysts for Carbon Capture, Sequestration and Valorisation, Process Biochemistry 46:1010.
27. Yadav, R., Joshi, M., Wanjari, S., Prabhu, C., Kotwal, S., Satyanarayana, T., Rayalu, S., 2012 Immobilization of Carbonic Anhydrase on Chitosan Stabilized Iron Nanoparticles for the Carbonation Reaction, Water Air Soil Poll 223:5345.
28. Rayalu, S., Yadav, R., Wanjari, S., Prabhu,C., ,Mushnoori, S., Labhsetwar, N., Satyanarayanan, T., Kotwal, T.,Wate, S.R., Sung-Gil Hong, JungbaeKim.Nanobiocatalysts for Carbon Capture, Sequestration and Valorisation Journal of Topics in Catalysis, 55 2013 1217-1221.
29. O'fagain, C., 2003, Introduction to the Field of Enzyme Immobilization and Stabilization Enzyme and Microbial Technology, 33:137.
30. Shanbhag, B.K., Liu, B., Fu, J., Haritos, V.S. and He, L., 2016. Self-Assembled Enzyme Nanoparticles for Carbon Dioxide Capture. Nano Letters, 16 (5): 3379–3384.
31. Khameneha, H.P., Bolouri, T.G., Nemati, F., Rezvania, F., ,Atter, F., Saboury, A.A. and Falahatia, M. 2017. A spectroscopic study on the absorption of carbonic anhydrase onto the nanoporous silica nanoparticle. International Journal of Biological Macromolecules, 99: 739-745.
32. Jia, H.F., Zhu, G.Y., Wang, P., 2003, Catalytic behaviors of enzymes attached to nanoparticles: the effect of particle mobility. Biotechnology and Bioengineering, 84 (4):406-414.
33. Chen, J.P., Su, D.R., 2001, Latex Particles with Thermo-Flocculation and Magnetic Properties for Immobilization of α-Chymotrypsin. BiotechnologyProgress, 17:369.
34. Kim, J., Grate, J.W., Wang, P., 2006.Nanostructures for enzyme stabilization Chemical Engineering Sciences, 61:1017.
35. Yan, M.,Ge, J., Liu, Z., Ouyang, P.K., 2006. Encapsulation of Single Enzyme in Nanogel with Enhanced Biocatalytic Activity and Stability. Journal of American Chemical Society 128:11008.
36. Kim, J, Grate, J.W., 2003,Single-Enzyme Nanoparticles Armored by a Nanometer-Scale Organic/Inorganic Network. NanoLett 3:1219.